

Figure 1

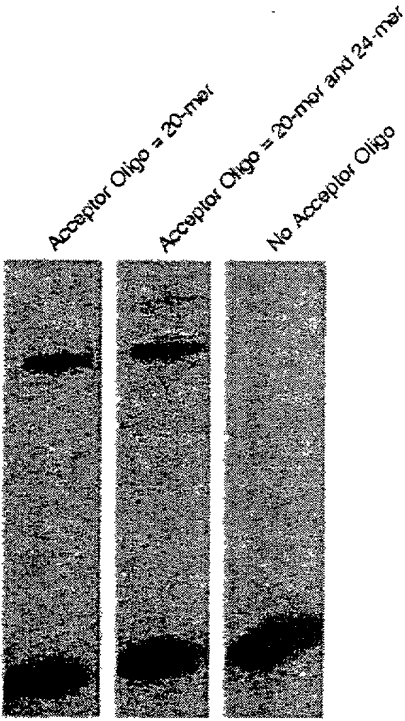


Figure 2

OPS at Topol cleavage site

↓

TCRIBamS: 5' AATTCGCGGCGCGCAAAAAGACTT-GATC 3'

TCRIBamAS: 3' GCGCCGGCGTTTTTCTGAA--CTAG--DMT 5'

Figure 3A

OPS at Topol cleavage site

↓

TCRBstBS: 5' AATTCGCGGCGCGCAAAAAGACTT-CG 3'

TCRBstBAS: 3' GCGCCGGCGTTTTTCTGAA--GC--DMT 5'

Figure 3B

TCRIBamS: 5' AATTCGCGGCGCGCAAAAAGACTTGATC 3'

TCRIBamAS: 3' GCGCCGGCGTTTTTCTGAACTAG 5'

Figure 3C

GATC removed on topo cleavage to give BamHI compatible 5' overhang

5' AATTCGCGGCGCGCAAAAAGACTTGATCAAGTCTTTTTGCGGCCGCG 5'

3' GCGCCGGCGTTTTTCTGAACTAGTTCAGAAAAACGCCGGCGCTTAA 3'

Figure 3D

Figure 3  
A - D

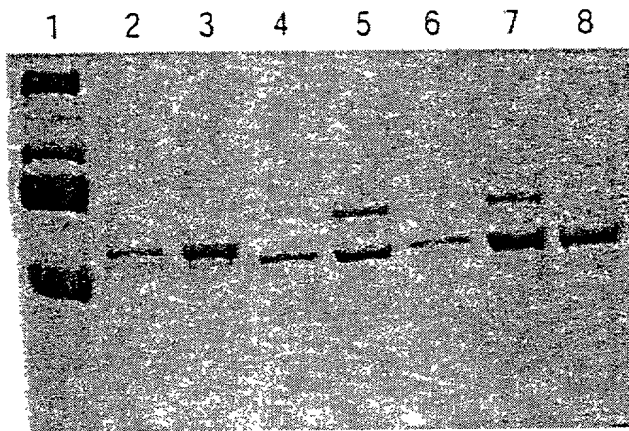


Figure 4

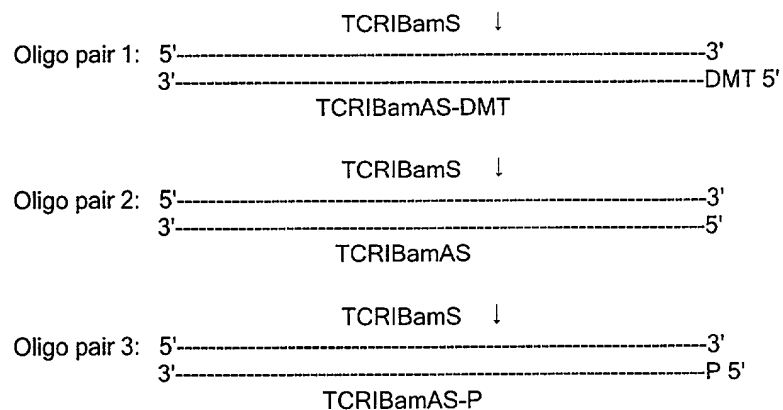


Figure 5A

"↓" indicates site of topo cleavage

Oligo pair 4: TCR1BstBS + TCRABstBAS-DMT

Oligo pair 5: TCR1BstBS + TCRABstBAS

Oligo pair 6: TCR1BstBS + TCRABstBAS-P

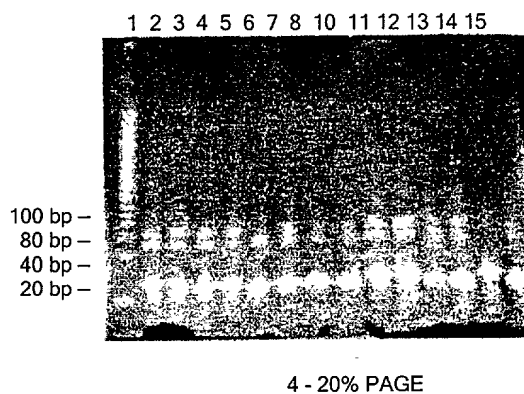


Figure 5B

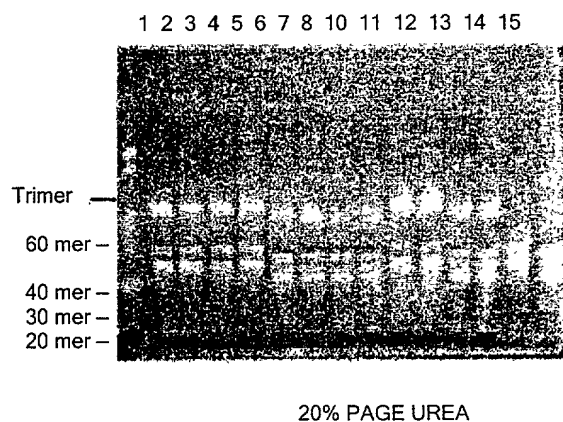
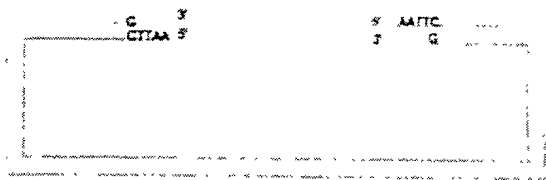


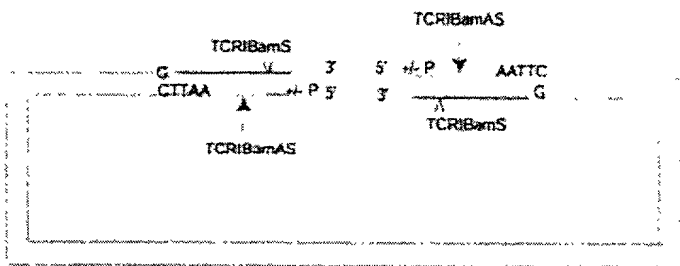
Figure 5C

Figure 5  
 A - C



pKSI linearized with EcoRI

Figure 6A



GATC overhang left after Topo cleavage and removal of 4bp downstream from Topo cleavage site

Adapted pKSI

site of Topo cleavage

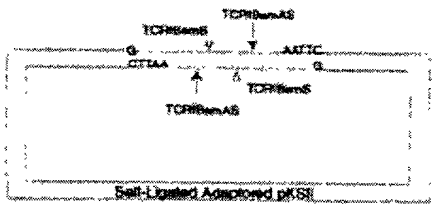


Figure 6B

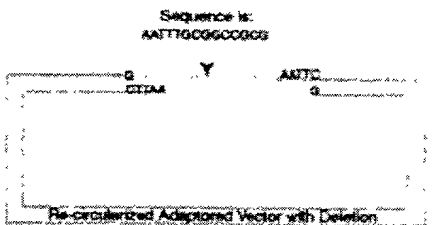


Figure 6C

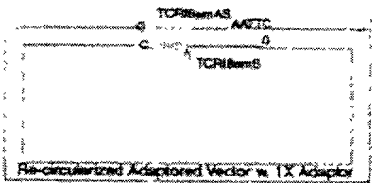


Figure 6D

Figure 6  
A - D

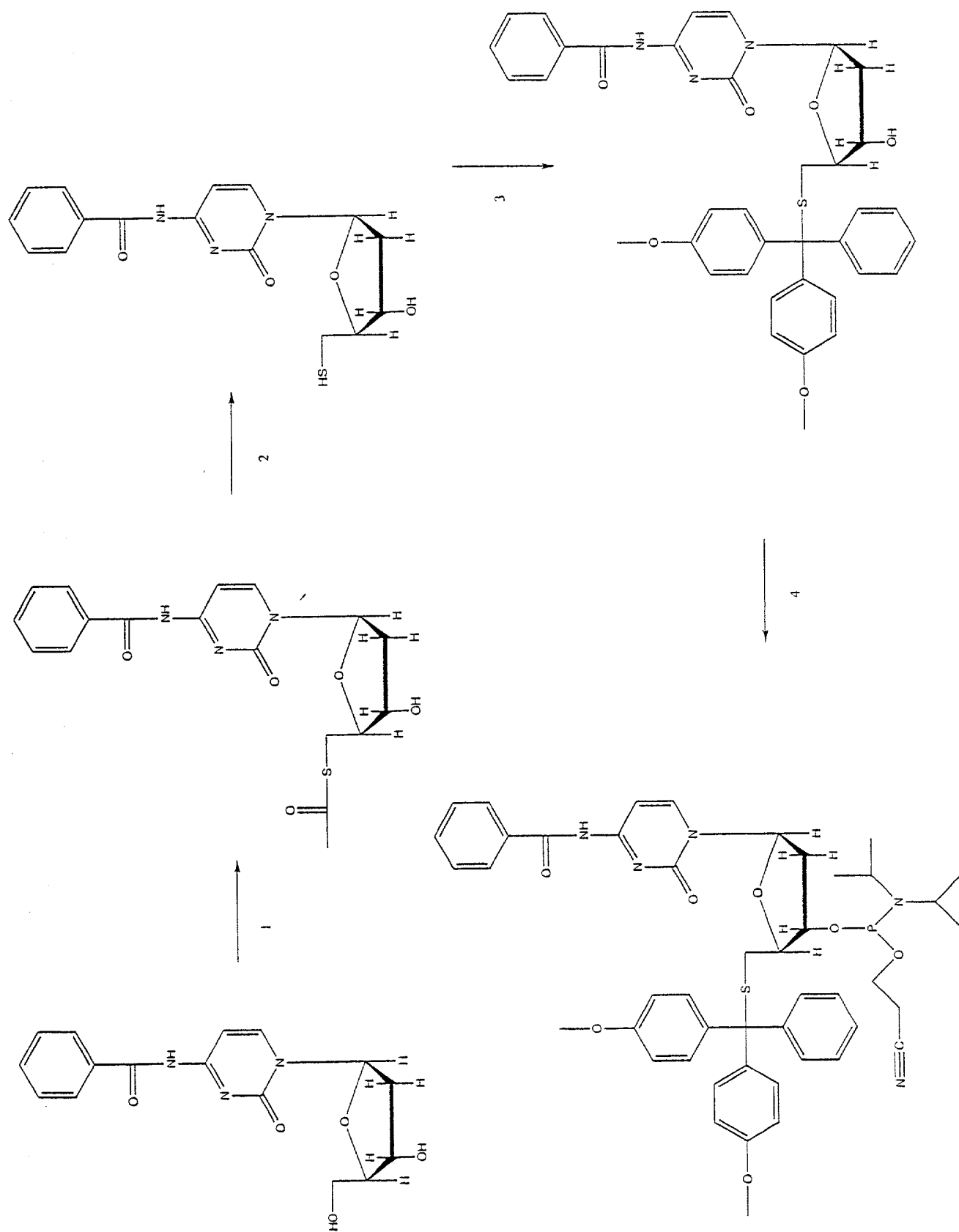


Figure 7A

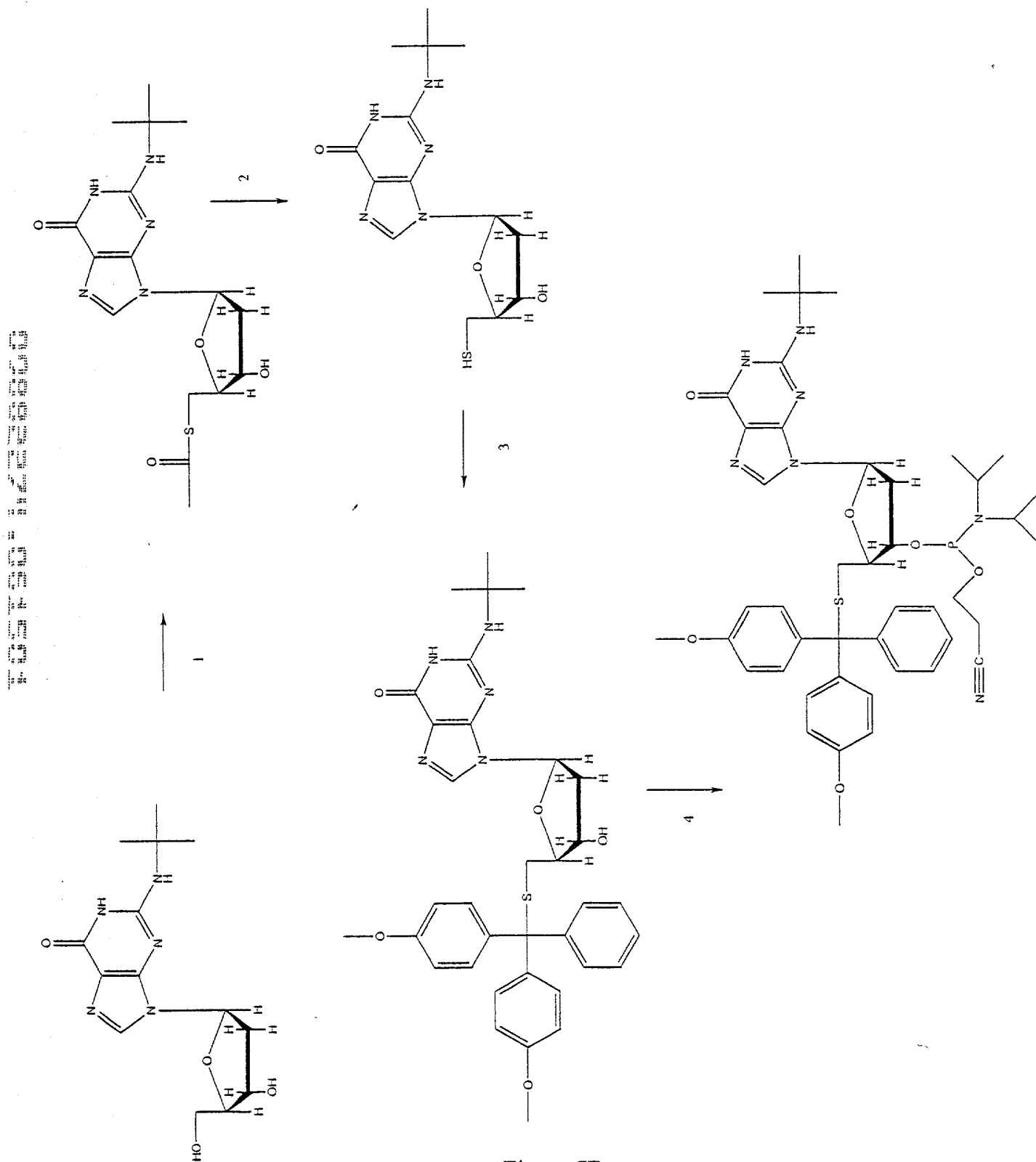


Figure 7B



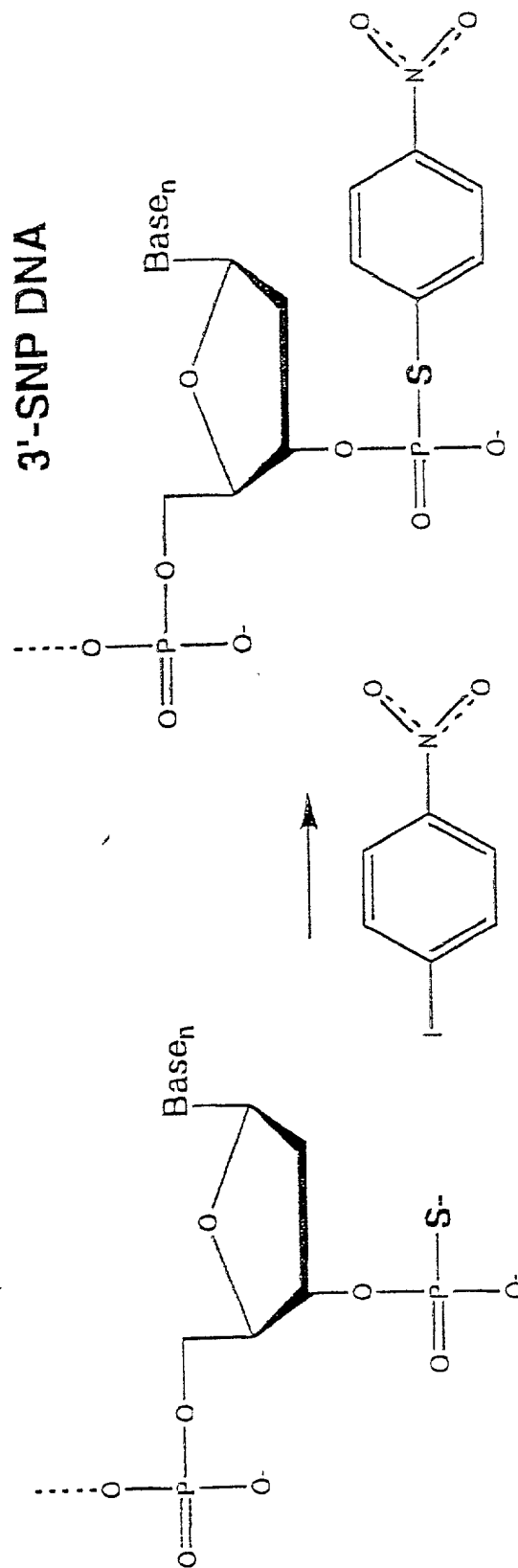


Figure 8